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Age and Developmental Stage Dependent Changes in Plasma Growth Hormone and Ghrelin Levels and Their Relationship in Normal and Short Stature Children

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Authors' Contribution
NK conceived and conducted the experiment and this particular study is part of her Ph.D. research work. GS helped in providing access to her patients and their data. MA and AAN helped in undertaking the research and analysis of data. SSRR and MQ designed the experiment, supervised the research work during its execution and helped NK in the write up of this manuscript.

Key words
Short stature, Ghrelin, Growth hormone, Short height, Delayed growth

ABSTRACT

In humans, growth is a complex mechanism that is controlled by growth hormone (GH), along with its mediator, insulin-like growth factor 1 (IGF-1) at each stage of development. The synthesis and release of GH is regulated by an intricate regulatory system involving GH releasing hormone (GHRH) and GH inhibiting hormone (GHIH) from the hypothalamus and ghrelin from the gastrointestinal tract. Disruption in any of the component of endocrine axis, i.e., the hypothalamus, ghrelin, GH or IGF-I, a mediator of GH action, may cause short stature. Short stature is defined as a height less than 2 standard deviation score (SDS) below the population mean. Since, ghrelin is the strongest stimulator of GH and previous studies did not find a significant relationship between GH and ghrelin in short stature children, therefore, the present study was designed to identify the levels of GH and ghrelin in normal and short stature children and determine the correlation between these hormones. Immunoradiometric assay (IRMA) was used for the analysis of plasma GH and enzyme linked immunosorbent assay (ELISA) was used for the analysis of plasma ghrelin in 84 normal school attending children and 35 short stature children of 2 to 14 years of age having decreased appetite. The levels of GH were significantly decreased in short stature children than controls while the mean plasma concentrations of ghrelin were significantly higher in short stature children as compared to control subjects of similar ages and developmental stages of infancy, prepuberty and early and mid-puberty. In addition, the levels of plasma ghrelin were positively correlated with plasma GH in normal subjects while negatively correlated in short stature children. In conclusion, this study demonstrated lower levels of GH but higher concentrations of ghrelin in short stature children and a positive correlation between GH and ghrelin in controls but a negative correlation in short stature children indicating a dysfunction of ghrelin and GH system of GH secretion.

INTRODUCTION

In humans, growth is a complex mechanism that is distinguished by the development of bone tissue and is controlled by multiple factors such as genetic, hormonal, nutritional, cultural and socio-economic conditions (Esposito *et al*., 2019; Ji *et al*., 2022). At each stage of development, the main hormone for statured growth is growth hormone (GH), along with mediator of its action, insulin-like growth factor 1 (IGF-1) (Esposito *et al*., 2019).

GH is released in the form of pulses and is controlled by an intricate regulatory system involving three hormones; two hypothalamic neuroendocrine hormones, GH releasing hormone (GHRH) and somatostatin or GH inhibiting hormone (GHIH) (Muller *et al*., 1999; Khatib *et al*., 2014); and a gastrointestinal hormone, ghrelin (Akalu *et al*., 2020). GHRH has a stimulatory effect on GH production and release while somatostatin has an

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inhibitory role (Muller *et al*., 1999). A third regulator of GH secretion is ghrelin, which markedly stimulates GH activity (Khatib *et al*., 2014).

Ghrelin is a unique peptide made up of 28 amino acid that, in humans, is secreted mainly by P/D1 cells of the stomach and in small amounts from small intestine, pancreas, kidneys, and other organs (Date *et al*., 2000; Mizutani *et al*., 2009; Akalu *et al*., 2020). It is one of the strongest known GH secretagogues (Lewinski *et al*., 2021) and is an additional element of GH/IGF-1 axis. Ghrelin was primarily discovered as an endogenic peptide able to bind to the GH secretagogue receptor (GHSR) in the brain, which is able to stimulate GH release from the anterior pituitary (Abdou *et al*., 2019).

Ghrelin occurs in two forms: des-acyl ghrelin (non-octanoylated form) and acyl ghrelin (octanoylated form). Eighty percent of ghrelin is in the form of des-acyl ghrelin, which is inactive and is not able to bind to GHSR. On the other hand, 20% of ghrelin is acyl ghrelin, i.e., the third carbon of ghrelin is octanoylated by ghrelin O-acyltransferase (GOAT) enzyme (Du *et al*., 2018; Akalu *et al*., 2020). Acyl ghrelin is active, binds to GHSR and activates different signaling pathways for GH production and secretion (Akalu *et al*., 2020).

Ghrelin is also a natural orexigenic factor, which increases the feeling of hunger resulting in increased food intake (Kojima *et al*., 1999; Lewinski *et al*., 2021). It is able to cross the blood-brain barrier and binds to GHSR present on the lateral hypothalamus of the brain, where it activates the neuropeptide Y/agouti-related peptide neurons as well as orexigenic neuropeptides, melanin concentrating hormone and orexin. This activates orexigenic pathways, inhibits anorexigenic pathways and stimulates food intake (Muller *et al*., 2015; Malhotra and Levitsky, 2021). In this way, ghrelin regulates body weight for a long time and is also involved in maintaining the glucose levels and immune system, and protection of the heart (Saranac and Gucev, 2016; Abdou *et al*., 2019). Therefore, ghrelin affects growth by increasing appetite and food intake and subsequent GH release (Abdou *et al*., 2019).

The GH molecule, thus produced, stimulates the liver for the synthesis of IGF-1 (Esposito *et al*., 2019). In addition, GH also acts directly on the cartilage cells in the growth plates of the long bones, causing the IGF-1 production at a local level (Baron *et al*., 2015; Esposito *et al*., 2019). Both GH and IGF-1 play important role in the regulation of linear growth (Abdou *et al*., 2019) by promoting the production and differentiation of young prechondrocytes and osteoblasts (Locatelli and Bianchi, 2014; Esposito *et al*., 2019). Moreover, GH also promotes the synthesis of new proteins and preserves the proteins already present in the cells (Khatib *et al*., 2014).

The GH release, similar to other pituitary hormones, is under feedback control. GH inhibits the secretion of GHRH and stimulates the release of GHIH by acting at the hypothalamic level, thus decreasing GH levels. Moreover, IGF-1 also suppresses GH secretion by directly acting on the pituitary gland. IGF-1 also increases GHIH secretion to inhibit GH release (Khatib *et al*., 2014).

Disruption in any of the component of endocrine axis i.e., the hypothalamus, ghrelin, GH or IGF-I may cause short stature (Zhou and Lv, 2021). A child is believed to be short, if he/she has a height more than 2 standard deviation score (SDS) below the population mean (Polidori *et al*., 2020). Since, ghrelin is the strongest stimulator of GH and no significant relationship was observed between GH and ghrelin in short stature children in previous studies (Norrelund *et al*., 2002; Muller *et al*., 2002; Avram *et al*., 2005; Espelund *et al*., 2005), therefore, the present study was designed to (i) find the GH and ghrelin concentrations in short stature children having decreased appetite and comparing them with age and developmental stage matched healthy control subjects. (ii) examine the correlation between GH and ghrelin in normal and short stature children.

MATERIALS AND METHODS

Subjects

Thirty-five children (21 boys, 14 girls) diagnosed with short stature visiting The Children's Hospital, Pakistan Institute of Medical Sciences (PIMS), Islamabad, were included in the current study. The ages of short stature children were between 2 and 14 years. Eighty-four healthy children (48 boys, 36 girls) of similar ages constituted the control group. The history of the subjects and their related information was documented in the especially developed questionnaire. Both control and short stature children were categorized into different age groups and different developmental stages including infancy (1-2 years), prepuberty (3-8 years for boys and 3-7 years for girls), early puberty (9-12 years for boys and 8-11 years for girls) and mid-puberty (13-14 years for boys and 12-13 years for girls) (en.wikipedia.org; www.healthline.com).

Inclusion criteria: In this study, the diagnostic criteria for the subjects included short stature i.e., height less than third centile or more than 2 SDS below the population mean. The short stature children having no history of chronic illness or medication use, birth weight proper for gestational age and not suffering from significant health problems were included in the study.

Exclusion criteria: The short stature children having chronic illnesses such as anemia, kidney or liver disease, achondroplasia, celiac disease etc. and those who were

suffering from syndromic conditions like Noonan's syndrome, Prader-Willi syndrome, Down's syndrome etc. were not included in this study.

Sample collection

From each normal and short stature child, approximately 2 mL sample of blood was drawn from antecubital vein for the analysis of hormonal levels in the plasma. The sample was put in gel tubes, immediately transferred to the lab, where it was spun for 15 min at 3000 rpm. This separated the plasma from other blood components, which was collected in Eppendorf tubes and was frozen at -20 °C until analyzed. Since, hormones are secreted in a diurnal pattern, therefore, blood sampling was restricted between 9 in the morning and 12 noon, so that the diurnal variations can be reduced.

Analysis of hormonal levels

The levels of hormones in the plasma samples were measured at Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad. Immunoradiometric assay (IRMA) (Cisbio Bioassays, France) was used for the analysis of plasma GH having a lower detection limit of 0.1 mIU/L. The intra-assay coefficient of variation (CV) was found to be ≤2.7% and the inter-assay CV was found to be ≤7.1%. The plasma concentrations of total ghrelin were evaluated by using a commercial Human Ghrelin ELISA Kit (Cusabio Biotech Co., USA). The lower detection limit of the kit was 0.156 pg/mL, and the intra-assay CV was ≤8% and the inter-assay CV was ≤10%.

Statistical analysis

The techniques such as Student's t-test, ANOVA and Pearson correlation r were employed for the statistical investigation of the data. All values were expressed as Mean±SEM (standard error of mean).

RESULTS

Age related changes in the mean plasma GH levels in normal and short statured boys and girls

Boys

The variations in the mean GH levels in normal and short stature boys from 2 to 14 years of age are shown in Table I. The mean GH levels were markedly lower in short stature boys than normal boys in all age groups from 2 to 14 years. In normal boys, as compared to the plasma levels at 2 years, the mean GH levels significantly decreased at 4 and 5 years of age. The mean GH levels slightly increased at 6, decreased at 7 and again significantly increased at 8 years of the life in normal boys. Furthermore, the mean GH concentrations in normal boys slightly decreased at 9 and then increased at 10-14 years of age. In short stature boys, the mean plasma GH concentrations decreased from 2 to 4 and slightly increased at 5 and 6 years of age. Furthermore, the mean GH levels slightly increased at 7, markedly decreased at 8, noticeably increased at 9, decreased at 10, increased at 11, markedly decreased at 12 and then slightly increased at 13 and 14 years of age in short stature boys.

Girls

The fluctuations in the mean levels of GH in normal and short stature girls from 3 to 13 years are presented in Table I. The mean levels of GH were higher in normal girls than short stature girls in all age groups from 3 to 13 years. In normal girls, as compared to the plasma concentrations at 3 years, the mean levels of GH were low at 4 and 5, increased at 8 and decreased at 9 years of age. In addition, the mean levels of GH in normal girls non-significantly increased at 10 and 11, significantly increased at 12 and further increased non-significantly at 13 years of life. In short stature girls, the mean levels of GH declined from 3 to 4, slightly increased at 5 and decreased at 8 and 9 years of age. Furthermore, the mean levels of GH slightly increased at 10, 11, 12 and 13 years of age in short stature girls.

Development related changes in the mean GH levels in normal and short statured boys and girls

Boys

The variations in the mean GH levels in normal and short stature boys at different stages of development are shown in Table II. The mean GH levels in normal boys significantly decreased from infancy to prepuberty but significantly increased at early and mid-puberty. In short stature boys, the mean GH levels slightly increased from infancy to prepuberty, non-significantly increased at early and non-significantly decreased at mid-puberty.

The mean concentrations of GH in plasma were markedly higher in normal boys as compared to short stature boys at infancy. Moreover, the mean GH levels in normal boys were significantly higher than short stature boys at prepuberty, and early and mid puberty.

Girls

The variations in the mean levels of GH in control and short stature girls at different stages of development are presented in Table II. The mean GH levels in normal girls slightly increased from prepuberty to early and significantly increased at mid puberty. In short stature girls, the GH levels non-significantly decreased from prepuberty to early but non-significantly increased at mid puberty.

Table I. Age related changes in the mean plasma GH and ghrelin levels (Mean±SEM) in normal and short stature boys and girls.

Age groups (years)	Plasma GH (mIU/L)		Plasma ghrelin (pg/mL)	
	Controls (n=4)	Short stature (n=35)	Controls (n=4)	Short stature (n=35)
Boys				
2	2.23±0.25	0.27±0 (1)	136.71±11.34	264.8±0 (1)
4	1.79±0.26	0.19±0 (1)	118.93±7.82	255.3±0 (1)
5	1.53±0.13	0.25±0.13 (2)	107.47±9.16	259.65±7.65 (2)
6	1.57±0.19	0.26±0 (1)	105.07±6.38	245.8±0 (1)
7	1.42±0.23	0.44±0.09 (3)	114.89±7.55	243.23±9.42 (3)
8	1.72±0.28	0.20±0.06 (2)	101.81±5.45	252.4±9 (2)
9	1.52+0.16	0.41±0.02 (2)	93.77±9.81	245.7±11.4 (2)
10	2.05±0.18	0.28±0.06 (2)	102.54±6.31	243.75±12.55 (2)
11	2.39±0.25	0.42±0.13 (2)	111.41±11.54	249.23±5.87 (2)
12	3.31±0.15	0.12±0.09 (2)	118.62±6.82	253.89±6.74 (2)
13	4.26±0.19	0.16±0.11 (2)	125.32±8.02	248.82±9.27 (2)
14	4.57±0.25	0.23±0 (1)	135.6911±9.92	242.87±0 (1)
Girls				
3	2.02±0.35	0.35±0 (1)	145.82±11.83	253.61±0 (1)
4	1.63±0.46	0.25±0 (1)	112.93±6.23	271.46±0 (1)
5	1.55±0.12	0.3±0 (1)	104.71±6.94	259.49±0 (1)
8	1.75±0.39	0.2±0 (1)	98.33±5.38	255.17±0 (1)
9	1.28±0.26	0.05±0.03 (2)	92.37±6.35	265.67±0.96 (2)
10	1.98±0.17	0.25±0 (1)	103.26±6.41	252.37±0 (1)
11	2.51±0.27	0.33±0.03 (3)	120.54±3.89	259.4±7.69 (3)
12	4.03±0.25	0.37±0.02 (3)	126.62±6.31	250.78±3.07 (3)
13	5.19±0.32	0.38±0 (1)	138.31±7.67	240.61±0 (1)

Table II. Developmental stage related changes in the mean plasma GH and ghrelin levels (Mean±SEM) in normal and short stature boys and girls.

Developmental stages	Plasma GH (mIU/L)		Plasma Ghrelin (pg/mL)	
	Controls (n)	Short stature (n)	Controls (n)	Short stature (n)
Boys				
Infancy	2.23±0.25 (4)	0.27±0 (1)	215.52±8.97 (4)	264.8±0 (1)
Pre-puberty	1.81±0.04 (20)	0.29±0.05 (9)	118.47±4.12 (20)	250.54±4.07 (9)
Early puberty	2.45±0.13 (16)	0.31±0.06 (8)	109.64±3.61 (16)	248.14±3.90 (8)
Mid puberty	4.42±0.09 (8)	0.18±0.07 (3)	130.23±3.44 (8)	246.83±5.71 (3)
Girls				
Pre-puberty	1.73±0.15 (16)	0.35±0.03 (3)	121.15±12.56 (16)	261.52±5.25 (3)
Early puberty	1.89±0.23 (12)	0.21±0.06 (7)	104.57±5.43 (12)	258.58±3.23 (7)
Mid puberty	4.41±0.41 (8)	0.37±0.01 (4)	130.50±4.12 (8)	248.24±3.34 (4)

The mean plasma GH levels were significantly higher in normal girls as compared to short stature girls at prepuberty, and early and mid-puberty.

Age related changes in the mean concentrations of ghrelin in normal and short statured boys and girls

Boys

The changes in the mean concentrations of ghrelin in

normal and short stature boys of different age groups are shown in Table I. The current investigation revealed higher concentrations of ghrelin in short stature boys than age matched controls in all age groups from 2 to 14 years. In normal boys, as compared to the plasma concentrations at the age of 2 years, the mean ghrelin levels non-significantly decreased from 4 to 6 years of age. Furthermore, the mean plasma ghrelin levels in normal boys non-significantly increased at 7 and slightly decreased at 8 and 9 years of life. Afterwards, the mean ghrelin levels non-significantly increased from 10 to 14 years of age in normal boys. In short stature boys, the mean ghrelin levels decreased from 2 to 4, showed a slight increase at 5 and then slightly decreased at 6 and 7 years of life. The ghrelin levels in short stature boys showed a slight increase at 8 but decreased at 9 and 10 years of age. The mean ghrelin levels in short stature boys slightly increased at 11 and 12 and then decreased at 13 and 14 years of age.

Girls

The changes in the mean concentrations of ghrelin in normal and short stature girls in relation to their age are shown in Table I. The mean concentrations of ghrelin were augmented in girls having short stature when compared with controls in all age groups from 3 to 13 years. It was observed that as compared to the plasma concentrations at the age of 3 years, the mean ghrelin concentrations in normal girls significantly decreased at 4 and non-significantly decreased at 5, 8 and 9 years of life. Moreover, the mean plasma ghrelin concentrations in normal girls non-significantly increased from 10 to 13 years of age. In short stature girls, the mean concentrations of ghrelin slightly increased from 3rd to 4th, decreased at 5th and 8th, slightly increased at 9th and again decreased at 10th year of life. The mean plasma ghrelin levels in short stature girls slightly increased at 11th and then decreased at 12th and 13th year of age.

Development related changes in the mean concentration of ghrelin in normal and short statured boys and girls

Boys

The mean ghrelin levels in normal and short stature boys at different developmental stages are shown in Table II. The mean ghrelin levels in normal boys significantly decreased from infancy to prepuberty, non-significantly decreased at early but significantly increased at mid-puberty. In short stature boys, the ghrelin levels slightly decreased from infancy to prepuberty, and early and mid-puberty.

The mean ghrelin levels were significantly higher in short stature boys than normal boys at different developmental stages of infancy, prepuberty, and early and mid puberty.

Girls

The variations in the mean concentrations of ghrelin in normal and short stature girls at different developmental stages are presented in Table II. The concentrations of ghrelin in normal girls significantly decreased from prepuberty to early but significantly increased at mid puberty. In short stature girls, the average plasma ghrelin levels non-significantly decreased from prepuberty to early and mid puberty.

The mean concentrations of ghrelin were significantly augmented in short stature girls than normal girls at different developmental stages of prepuberty, and early and mid puberty.

Correlation between GH and ghrelin levels in normal and short stature boys and girls at different developmental stages

A positive correlation was observed between the plasma GH and ghrelin levels in normal boys at infancy, prepuberty and mid puberty but a negative correlation was witnessed at early puberty. On the other hand, in short stature boys, the correlation between the plasma GH and ghrelin levels was negative at prepuberty and early but positive at mid puberty.

Likewise, there was a strong positive correlation between the plasma GH and ghrelin levels in normal girls at prepuberty and mid puberty but a negative correlation was observed at early puberty. In contrast, in short stature girls, the correlation between the plasma GH and ghrelin levels was negative at prepuberty and mid but positive at early puberty.

DISCUSSION

In the current investigation, the GH and ghrelin levels in the plasma were measured and the relationship between the two hormones was determined in normal and short stature children having decreased appetite. Our results identified significantly lower GH levels in short stature boys and girls of all ages from 2 to 14 years and at all developmental stages of infancy, prepuberty, and early and mid-puberty as compared to age and developmental stage matched normal children indicating GH deficiency in all short stature children. A recent study also reported that insufficient secretion of GH results in the dysfunction of endocrine axis and causes short stature (Zhou and Lv, 2021). Thus, proper synthesis, release and function of GH is vital for the physiological growth and optimal functioning of the human organism (Ogilvy-Stuart, 2003; Hage *et al*., 2021; Plachy *et al*., 2022). Since, GH is

secreted in pulses and a single measurement of GH levels in the blood is not sufficient, GH stimulation test and/or IGF-1 concentrations are more important (Bright *et al.*, 2021). In the present study, we were unable to perform GH stimulation test or IGF-1 for all of the short stature children due to economic constraints. But some children were randomly selected for GH stimulation test using L-dopa and some for IGF-1 levels. The L-dopa stimulation test showed blunted GH secretion and the results of IGF-1 were found to be very low. Based on the results obtained, all short stature children were characterized as being GH deficient.

The present study demonstrated significantly higher ghrelin levels in short stature boys and girls than age matched normal subjects between 2 and 14 years of age. In the same way, the mean ghrelin levels in short stature boys and girls were significantly higher in different developmental stage groups as compared to normal boys and girls of same developmental stage. In line with our observations, some recent studies have also shown significantly higher ghrelin concentrations in the morning in Egyptian and Polish idiopathic short stature (ISS) and GH deficient children (Taha, 2019; Stawerska *et al.*, 2020). In a similar manner, some previous studies have also reported significantly higher levels of ghrelin in Polish short thin children (Stawerska *et al.*, 2017), malnourished Turkish children (Erdemir *et al.*, 2016), Chilian ISS children (Iniguez *et al.*, 2011) and Turkish short stature children with constitutional delay in growth and puberty (CDGP) (Camurdan *et al.*, 2006) as compared to normal children. Moreover, higher ghrelin levels were also observed in short stature children having partial GH deficiency as compared to normal subjects (Wang *et al.*, 2019). Some other studies observed that the ghrelin levels in short stature children without GH deficiency were associated with the body mass index (BMI) of the children. Higher levels of ghrelin were evidenced in short stature slim children as compared to the obese ones (Stawerska *et al.*, 2017; Lewinski *et al.*, 2021). However, in our study, higher ghrelin levels were observed in short stature children having higher BMI i.e., overweight and obese. Additionally, a previous study on short stature children having different etiologies (GH deficiency, ISS and neurosecretory dysfunction) reported that the fasting levels of ghrelin were higher in children having lower IGF-1/insulin-like growth factors binding protein-3 (IGFBP-3) ratios, which is a useful way of finding overall GH levels (Stawerska *et al.*, 2012). Therefore, it was suggested that lower IGF-1 bioavailability and hence lower GH levels stimulates the synthesis and release of ghrelin (Stawerska *et al.*, 2012; Lewinski *et al.*, 2021). Likewise, another previous study assessed the levels of ghrelin, IGF-1 and IGFBP-3 in children having CDGP type of short stature and discovered higher concentrations of all three hormones as compared to normal children (Camurdan *et al.*, 2006). The study further reported that weight and height were independent negative determinants of ghrelin concentrations (Camurdan *et al.*, 2006). Thus, it was concluded that lower weight or lower height represents a poor nutritional state, which leads to an increase in ghrelin levels (Camurdan *et al.*, 2006; Perchard and Clayton, 2017).

In contrast to higher concentrations of ghrelin in short stature children of our investigation, a recent study found a non-significant difference between mean concentrations of ghrelin in Egyptian normal and short stature underweight children (Abdou *et al.*, 2019). Similarly, some previous studies also demonstrated non-significant difference in total ghrelin levels in Iranian ISS children (Razzaghy-Azar *et al.*, 2015), short stature USA children (Pinsker et al., 2011) and Turkish children with CDGP (Sen *et al.*, 2010) as compared to normal children. Moreover, Engstrom *et al.* (2003) also found normal levels of ghrelin in GH deficient patients. In our investigation, we identified higher ghrelin levels in short stature children having decreased appetite, which may suggest that dysfunctional ghrelin receptor or downstream ghrelin signaling pathways may result in lower GH levels and absence of negative feedback on the secretion of ghrelin in short stature children.

Our study identified a positive correlation between the plasma GH and ghrelin concentrations in normal children while a negative correlation in short stature children. The positive correlation in normal children observed in this study indicates that ghrelin affects the secretion of GH. Ghrelin directly stimulates GHRH and indirectly inhibits GHIH, which in turn, increases GH secretion (Andrich *et al.*, 2012; Saranac and Gucev, 2016; Akalu *et al.*, 2020). Some recent studies on animals also reported that after injection of ghrelin, the levels of GH in the plasma as well as the mRNA levels of GH in the pituitary, significantly increased indicating a positive correlation between GH and ghrelin (Ogawa *et al.*, 2018; Akalu *et al.*, 2020). Furthermore, studies on healthy young men identified a significant correlation between GH and the mean acyl-ghrelin concentrations (Nass *et al.*, 2008, 2011). However, a number of clinical studies on healthy individuals are available in the literature, which present conflicting results (Nass *et al.*, 2011). These studies showed a positive relationship between GH and circulating ghrelin levels (Koutkia *et al.*, 2004), found no relationship between GH and ghrelin (Norrelund *et al.*, 2002; Avram *et al.*, 2005; Espelund *et al.*, 2005), and identified a relationship between GH and ghrelin that is dependent on certain circumstances like fasting or the time according to the clock (Muller *et al.*, 2002).

We examined that GH and ghrelin were negatively correlated in short stature children, a finding supported by another study, which also stated a negative correlation between GH and ghrelin levels in Egyptian ISS and GH deficient children (Taha, 2019). Likewise, Stylianou *et al*. (2011) also showed a negative relationship between circulating GH and ghrelin concentrations in Greek short stature children having normal GH. In contrast, Abdou *et al*. (2019) did not find any correlation between total ghrelin levels and IGF-1, a mediator of GH, in nutritionally stunted Egyptian children. Moreover, some previous studies also reported no correlation between ghrelin and GH and IGF-1 levels in Italian and Iranian children with GH deficiency and ISS (Prodam *et al*., 2012; Razzaghy-Azar *et al*., 2015).

Many *in vivo* and *in vitro* experiments depict that GH and ghrelin systems are clinically relevant as these studies presented that ghrelin strongly stimulated GH levels in a manner that is dependent on dose of ghrelin (Bitar and Coy, 1991; Sattler, 2013; Khatib *et al*., 2014). It was demonstrated that when ghrelin was administered intravenously, peak GH levels were observed after 5-15 min of the injection, which returned to normal levels after 1 hour (Strasser *et al*., 2008; Khatib *et al*., 2014). Moreover, *in vitro* studies on primary pituitary cells demonstrated that ghrelin only stimulates GH release, without affecting other four hormones of the pituitary (Redman *et al*., 2010; Khatib *et al*., 2014), thereby suggesting that ghrelin acts directly on pituitary somatotropes. Therefore, it has been suggested that altered levels of total ghrelin or acylated ghrelin or ghrelin receptor structure or function may affect growth by influencing GH secretion and IGF-1 production or by altering appetite and food intake (Pinsker et al., 2011).

CONCLUSION

In conclusion, we demonstrated lower GH and higher ghrelin levels in children with short stature than the normal ones. We also showed a position correlation between GH and ghrelin in normal but a negative correlation between the two in short stature children.

DECLARATIONS

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IRB approval

Ethics Committee for Use of Human Subjects of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan approved the study.

Informed consent

An informed written consent was obtained from patients and/or their guardians.

Statement of conflict of interest

The authors have declared no conflict of interest.

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